Nigerian Research Journal of Chemical Sciences **(ISSN: 2682-6054)**
Volume 8, Issue 1, 2020

Bacteriological Assessment of Some Streams in Nsu Ehime Mbano Local Government Area of Imo State, Nigeria

[1]Asionye, E.I., [2]Ndukwe, N.N., [2]Tyohemba, S.T., [2]Memi, G.G.

[1]Department of Environmental Management and Pollution Control, Nigerian Maritime University (NMU), Okerenkoko, Delta State, Nigeria

[2]Department of Biological Sciences, Faculty of Sciences, Federal University, Kashere, Gombe State, Nigeria

Corresponding Author: nelson_ndukwe@yahoo.com

ABSTRACT

This study presented the bacteriological quality of three most used surface water sources in both wet and dry season (Nkwo, Dorogo and Acha) in Nsu Ehime Mbano Local Government Area (LGA) of Imo State, Nigeria. Global positioning system was used for geo-locating the sampling sites. The samples were collected and transported to the laboratory twice in a month using standard procedures for analyses. Bacteriological analyses carried out were: Total heterotrophic bacterial counts, Total coliform counts, Faecal coliform and other water pathogens. Biochemical test for characterization was done to identify the isolates. The total heterotrophic bacterial counts of Nkwo, Dorogo and Acha stream water samples for wet season ranged from 3.249198, 3.30103 and 3.40654 $\log_{10}$CFU/ml respectively and lower in values during dry season; 3.070038, 3.164353 and 3.40654 $\log_{10}$CFU/ml, respectively. The total coliform of the three streams ranged from 22.5, 26.5 and 47 MPN/100ml in the wet season and during the dry season were 19.75, 21.75 and 33.5 MPN/100ml respectively. The faecal coliform counts of Nkwo, Dorogo and Acha water samples ranged from6.700, 13.55 and 28.25 MPN/100ml in the wet season and during the dry season were 6.45, 11.45 and 21.50 MPN/100ml respectively. Nine bacteria genera were isolated and identify from the study which were *Escherichia* sp., *Salmonella* sp., *Shigella* sp., *Citrobacter* sp., *Proteus* sp., *Klebsiella* sp., *Vibrio* sp., *Bacillus* sp. and *Enterobacter* sp. This study revealed poor bacteriological quality of the streams, as pathogenic microorganisms (*Salmonella* sp., *Shigella* sp. and *Vibrio* sp.) of health significance were isolated from these streams. Therefore, treatment is needed before the consumption of these waters, especially during the wet season. The cheapest and safest local purification methods like boiling and

filtration available to the rural dwellers, are therefore recommended for the study area as the values obtained are not incompliance with World Health Organisation (WHO) and Standard organisation of Nigeria (SON) guidelines for water intended for domestic use.

Keywords: Coliforms, enteric pathogens, heterotrophic bacteria, water.

INTRODUCTION

Water used for drinking and other domestic purposes in most communities in developing countriesare obtained from natural sources such, as rivers, streams, lakes, ponds and springs, or artificial sources such as wells and boreholes. Most times these water supplies, particularly, streams, rivers, lakes, ponds and wells are likely to be polluted with wastes.

The microorganisms in these natural sources are numerous in both numbers and diversity [1]. Microbial populations of the microbes found in surface waters comprise both indigenous and transient populations which result in contamination of the different water sources due to their exposure. Continuous abuse of water bodies have resulted to eutrophication with issues like the harmful algal blooms in most parts of the world. The ingestion of microbial and chemically contaminated water results in several disease outbreak or ill health and some cases of death. As the science of epidemiology evolved, so did suspicions about the healthiness of water bodies evolved. The bacteriological analysis of different sources of water plays a key role in assessment of the healthiness of water anywhere in world [2]. Based on the result of bacteriological and physicochemical analysis, water treatment works can optimize treatment process. The bacteriological and physicochemical qualities of most ground water, pipe borne water and other natural water sources in Nigeria have been reported to exceed the WHO standard for safe water in their categories [3]. Microbiological media are used to isolate and identify microorganisms. This improvement has been harnessed for the isolation of waterborne pathogens. In some cases where these techniques are not enough for the identification of the organism, direct microscopy, conventional biochemical tests, molecular Analysis and Kits are available for their identification. The Most Probable Number (MPN) enumerates the level of microbial contaminants. A more advanced method of identifying an organism is the molecular identification of the isolated organism using a recombinant DNA technology, which make use of a gene probe. The goal of World Health Organization is that water should be free fromcontaminants [4]. All assessed water qualities, are always compared with the World Health

Organization standards to know the healthiness of that particular water category. If the analysed water body does not exceed the WHO standard, such water will not pose a health threat and is therefore safe. In the contrary, if the water exceeds the WHO standard for its category, such water pose a health risk and is therefore unsafe.

So many water bodies have been declared unsafe, while some are being treated for recreation purposes. Some drinking water are contaminated by toxic chemicals and pathogenic microorganisms to a level that exceeds the WHO standard. Public health scientists believed that there is a strong relationship between the level of contamination of water andthe risk of acquiring certain diseases and epidemics associated with certain uses of water. Due to the improper disposal of untreated wastes in the developing countries, with water bodies used as a free receptacle for wastes, water bodies such as drinking streams and natural pools are sources of pathogenic organisms which are acquired through their uses such as drinking and swimming [5]. Most enteric disease causing organisms are resident in such water bodies, hence could act as fomite for spreading diseases and epidemics.

This study is aimed at determining the bacteriological quality of some streams used for recreation and drinking in the study area; to ascertain the level of microbial contamination in both water sources in dry and wet season, and also to enumerate and identify the coliforms domiciled in the study area.

MATERIALS AND METHODS

Study Area

The area under investigation (Fig. 1) is Nsu autonomous community located in Ehime Mbano local government area of Imo state, south-east of Nigeria.It is a rural settlement and a nodal/linear settlement (Iwena, 2007) with coordinates representing various the sampling points (A and B):

Streams	Sample A (Nº)	Sample A (Eº)	Sample B (Nº)	Sample (Eº)
Nkwo Stream	N05º38’ 33.1’’	E007º21’ 01.2’’	N05º 38’33.7’’	E007º 21’00.8’’
Dorogo Stream	N05º38’ 35.7’’	E007º 20’ 56.0’’	N05º 38’35.1’’	E007º 20’57.3’’
Acha Stream	N05º 39’55.7’’	E007º 20’56.5’’	N05º 38’56.0’’	E007º20’56.9’’

It is highly populated with estimated population of around 30,000 people. Nsu is characterized by hills. Indigenes are mostly in small scale businesses and subsistence farming.

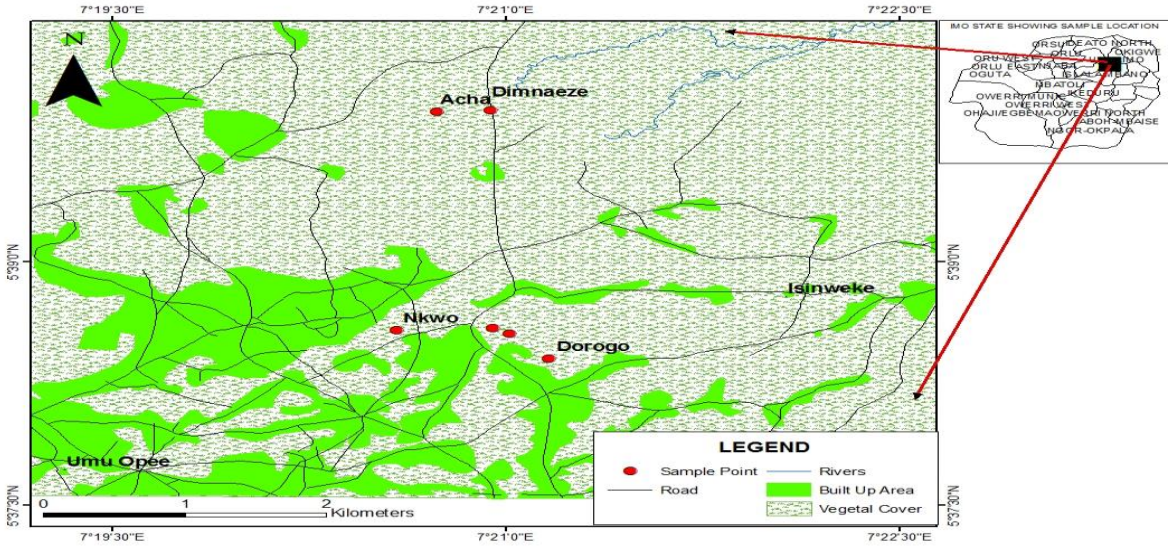

Figure 1: Map of Nsu Ehime Mbano Imo State showing the three study areas [6].

Samples Collection

Sterile bijour bottles were used all through the sample collection. The stream waters were collected directly from the same points where the public users fill their drinking containers. Eight samples were collected from each stream, four samples in the wet season (September and October, 2014) and four in the dry season (December, 2014 and January, 2015) respectively. Two samples (A and B) were collected every month from each stream. All samples were transported to the laboratory inside a well lagged cooler, within 5-7 hours. Recreational water samples (Acha Stream) were collected aseptically by placing the container below the water surface then moving it forward in a manner that prevented the water coming in contact with the hand from entering the bottles.

Sterilization

All glass wares, media, diluents and non-heat labile materials used in this work were sterilized using the autoclave. 95% of ethanol was used to sterilize the materials like working bench. Bunsen burner was used to sterilize inoculating materials.

Total Heterotrophic Bacteria Counts

The total heterotrophic count was done using nutrient agar. The medium was prepared according to the manufacturer's specification. It was poured into the petri dishes. An aliquot of 0.1 ml of the diluted samples was plated on the nutrient agar plates. The plates were incubated at 35 ^{o}C for 24-48 hours. The bacteria colonies were counted after the incubation. The colony forming units was estimated using the formula:

$$\frac{\text{Number of Colonies x Reciprocal of dilution}}{\text{Volume or Amount Plated}}$$

Enumeration of Total and Faecal Coliforms (MPN METHOD)

The most probable Number (MPN) technique was employed for the enumeration of coliform bacteria and faecal coliforms [7]. This method involved three major stages: the presumptive, confirmatory and completed test.

Presumptive Test

MacConkey broth was prepared in double strength and single strength. Three sets of 3 tests tubes were used. Three tubes contained the double strength and the other 6 tubes contained the single strength. Each tube was inserted with a Durham's tube for gas collection. 10ml of water sample was inoculated into each of the double strength tube. To a set of the single strength tube 1.0ml was inoculated. To a set of the other single strength 0.1ml was inoculated. The inoculated tubes were incubated at 35^{o}C for 24 to 48 hours. A change in the colours of the inoculated tubes from purple to yellow indicates a positive presumptive test. A gas collection with burbles In the Durhams tube was also an indicator of coliform presence.

Confirmatory Test

Eosine Methylene Blue (EMB) agar was used. A loopful of the positive tubes from the presumptive test was aseptically streaked on the EMB agar and incubated for 24-48 hours at 37 ^{o}C. After 24-48 hours plates were observed for the presence of colonies with green metallic sheen. The appearance of a green metallic sheen is a confirmation of coliform presence.

Completed Test

A loopful of the positive tubes from confirmatory test was inoculated into 10 ml of sterile lactose broth. Inoculated tubes were incubated for 24 hours at 37 ^{o}C. A change in colour of the broth and gas formation in the Durham's tube completed the test for coliform bacteria.

Faecal Coliform Test

Lactose broth from the completed test set of tube was incubated at about 43-44 ^{o}C for 24 hours. Acid and gas production at that incubation temperature and the change of the colouration to yellow indicated the presence of faecal coliforms.

Salmonella-Shigella Counts

Salmonella/shigella agar was prepared following producer's direction. The agar was allowed to gel. 0.1ml of each sample was dispensed on the sterile agar. Glass rod was used to spread the water sample on the SSA near a Bunsen burner flame. The plates were covered with a cling film and aluminum foil. It was incubated under 37 ^{o}C for 24-48 hours. The prepared agar was turbid and greenish-yellow. The appearance of black colonies indicates the presence of *Salmonella* sp. The appearance of clear, colourless and transparent-yellow colonies indicates the presence of *Shigella* sp

Isolation of *Vibrio* spp.

Thio – Sulphate Citrate Bile Salt Sucrose (TCBS) agar was prepared following the manufactures directions. The samples were streaked on the agar. The streaked plates were incubated at 35^{o}C for 18–24 hours. Culture was viewed immediately after removal from the incubator to avoid the reversion of the yellow colony colour of the *Vibrio* sp., to green at room temperature. The appearance of yellow colonies on the TCBS agar is an evidence of the *Vibrio* sp. presence.

Identification of Isolates

The water samples were inoculated on MacConkey agar using spread plate method. 0.1ml of the water samples was pipetted and spread with a sterile glass spreader. The plates were incubated at 37 ^{o}C for 24 hours. Morphological characteristics of the colonies were used for differentiating the organisms based on their growth criteria as listed in Cheesbrough [8]. Biochemical characteristics of the respective isolates were determined with the use of batteries of biochemical tests [9].

RESULTS AND DISCUSSION

Total heterotrophic bacterial counts

The seasonal variation in the total heterotrophic bacterial counts of the three surface waters in Nsu community is illustrated in Figure 2. For Nkwo stream, the total heterotrophic bacterial counts varied from 3.2491 log_{10}cfu/ml in the wet season months to 3.0700 log_{10}cfu/ml in the dry season months. For Dorogo stream, the total heterotrophic bacterial counts varied from 3.3010 log_{10}cfu/ml in the wet season to 3.1643 log_{10}cfu/ml in the dry season. For Acha stream, the total heterotrophic bacterial counts varied from 3.6459 log_{10}cfu/ml in the wet season months to 3.4065 log_{10}cfu/ml in the dry season. The graph shows that the water samples from three streams had their total heterotrophic bacterial counts above the SON and WHO standards for water being 2 log cfu/ml for both seasons.

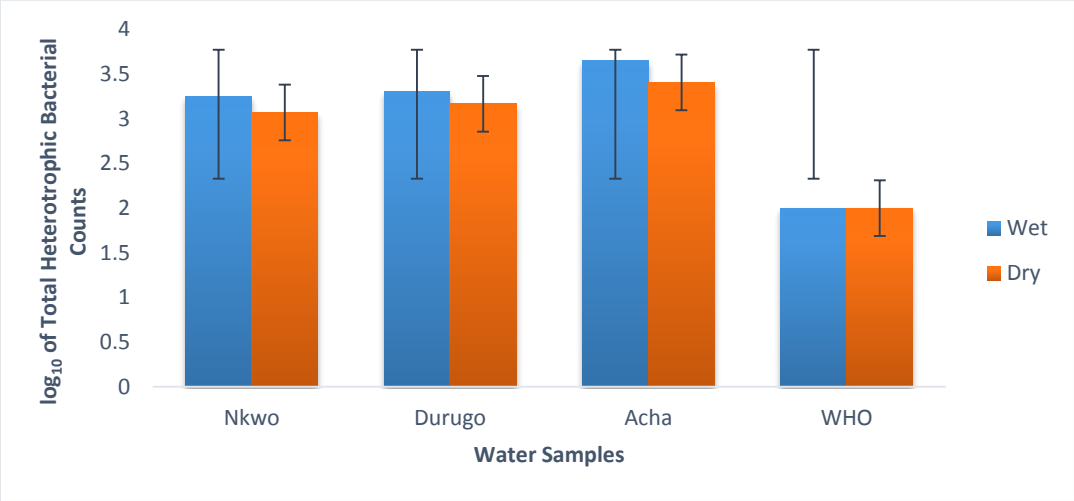

Figure 2.0: Log_{10}CFU/ml Seasonal Variation of Total Heterotrophic Bacteria Counts of the Surface Waters in Nsu for the Wet Season and Dry Season.

Biochemical characteristics of bacteria isolates from stream water samples in Nsu community

Table 1.0 shows the biochemical characteristics of the bacteria isolated from surface waters in Nsu community. The table revealed that both pathogenic bacteria and normal micro-flora were present in the samples. The table also revealed that Gram negative bacteria have about 80% predominance over Gram positive bacteria. The isolates were majorly enteric Gram negative of faecal-oral route.

Table 1: Biochemical Characteristics of the Bacteria Isolated from Surface Waters in Nsu community

Grm	Su	Ma	G	Ca	Ox	In	Ci	Mo	St	MR	VP	SI	B	Ga	H_2S	Isolates
-	+	+	+	+	-	-	-	+	+	+	-	A	A	+	-	*Escherichia* spp.
-	+	+	+	+	-	+	+	+	+	+	-	B	A	+	+	Salmonella spp.
-	-	+	+	+	-	-	-	-	-	+	-	B	A	-	-	*Shigella* spp.
-	+	+	+	+	-	+	+	+	-	+	-	B	A	+	+	*Citrobacter* spp.
-	-	+	+	+	-	-	-	+	+	+	-	B	A	+	+	*Proteus* spp.
-	+	+	+	+	-	+	-	-	-	-	-	B	A	+	-	*Klebsiella* spp.
-	+	+	+	+	-	-	+	-	-	-	+	B	A	+	-	*Vibrio* spp.
+	-	+	+	+	-	+	+	+	+	-	+	A	A	+	-	*Bacillus* spp.
+	+	+	+	+	-	-	+	+	-	-	+	B	A	+	-	*Enterobacter* spp.

Key: Grm=Gram's reaction, SU=Sucrose, Ma=maltose, G=glucose, Ca=Catalas, Ox=Oxidase, In=Indole, Ci=Citrate, Mo=Motility, St=Starch, MR=Methyl Red, VP=Voges-Proskaur, Sl=Slant, B=Butt, Ga=Gas, H_2s=Hydrogen Sulphide

Total coliform counts

The seasonal variation in the total coliform enumeration of the surface waters in Nsu for the Wet season and dry season are illustrated in Figure 3. For Nkwo stream, the total coliform varied from 22.25 MPN/100ml in the wet season to 19.75 MPN/100ml in the dry season. For Dorogo stream, the total coliform counts varied from 26.5 MPN/100ml in the wet season to 21.75 MPN/100ml in the dry season. For Acha stream, the total coliform counts varied from 47 MPN/ml in the wet season to 33.5 MPN/100ml in the dry season. The graph shows that the three stream water samples had their total coliform counts above the SON and the WHO standards for water being 0 MPN/100ml in both seasons.

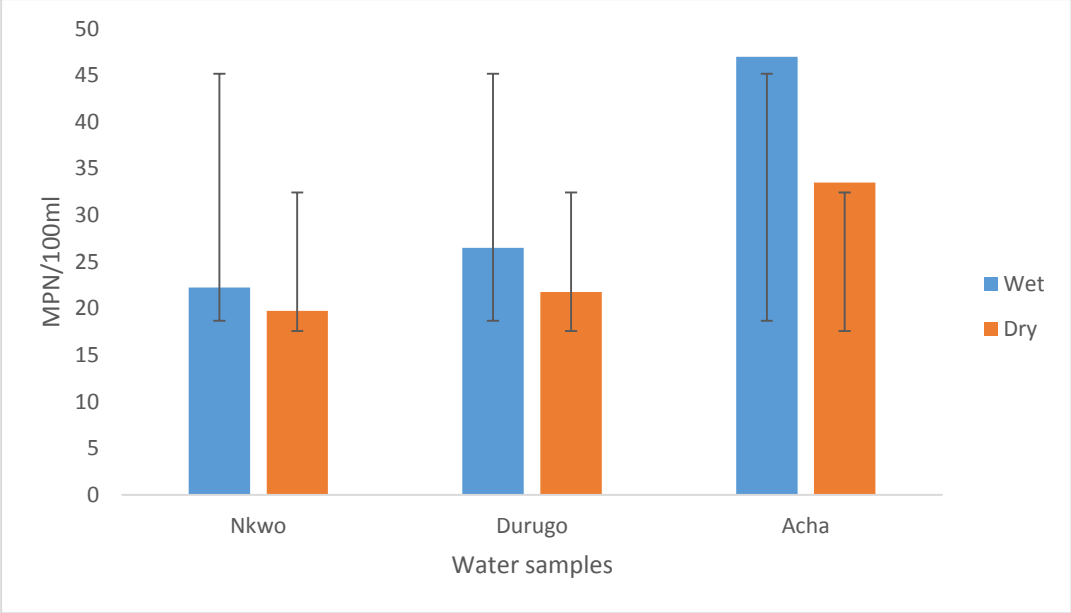

Figure 3.0: Mean Most Probable Number (MPN) Index of the Total Coliform Enumeration of the Surface Waters in Nsu for the Wet Season and Dry Season.

Faecal coliform counts

The seasonal variation in the faecal coliform enumeration of the surface waters in Nsu for the wet season and dry season respectively, are illustrated in Figure 4. For Nkwo stream, the Faecal Coliform counts varied from 6.7 MPN/100ml in the wet season to 6.45 MPN/100ml in the dry season. For Dorogo stream, the faecal coliform counts varied from 13.55 MPN/100ml in the wet

season to 11.35 MPN/100ml in the dry season. For Acha stream, the faecal coliform counts varied from 28.25 MPN/ml in the wet season to 21.25 MPN/100ml in the dry season. The graph shows that the three stream water samples had their faecal coliform counts above the SON and the WHO standards for water being 0 MPN/100ml in both seasons

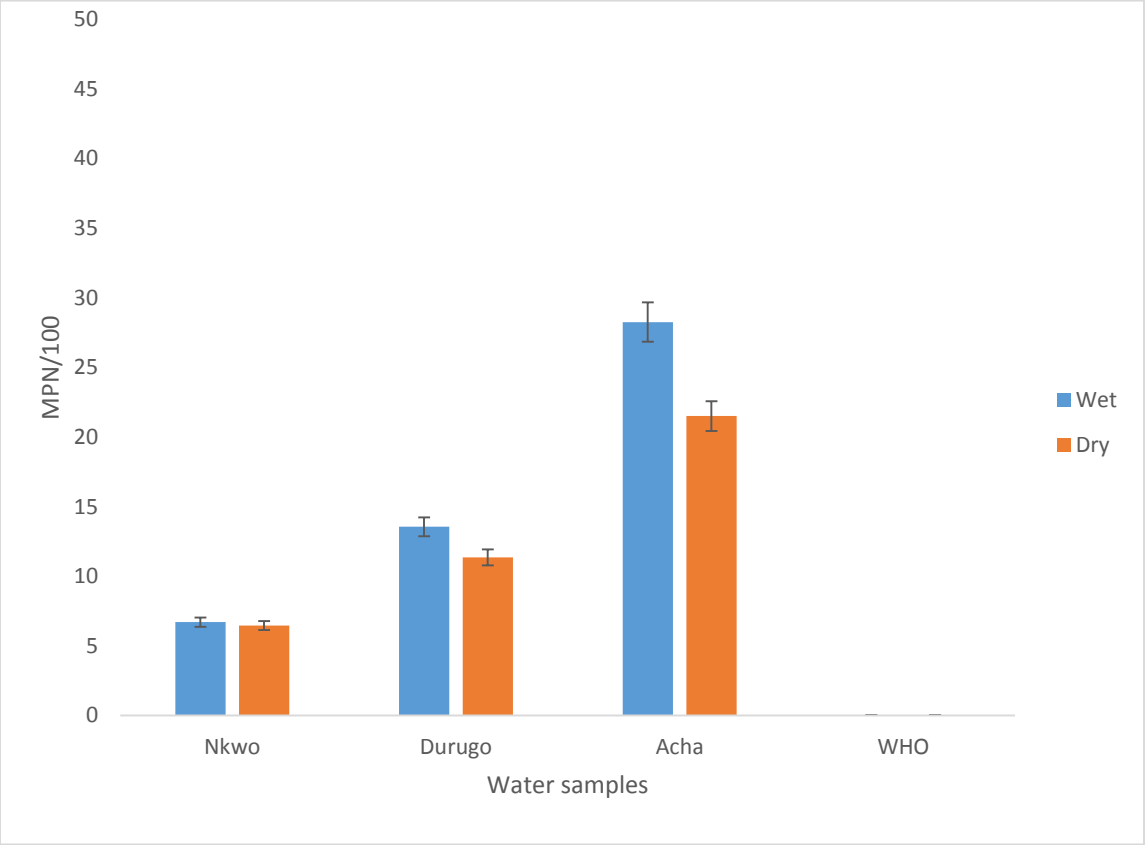

Figure 4.0: Faecal Coliform Enumeration of the Surface Waters in Nsu for the Wet Season and Dry Season

***Salmonella* counts**

Figure 5.0 illustrates the results for the seasonal variations in the *Salmonella* counts of the three streams in Nsu community. For Nkwo samples, the *Salmonella* counts varied from 1.39794 $\log_{10}$cfu/ml in for the wet season to 0 $\log_{10}$cfu/ml for the dry season months. Dorogo water samples had 2 $\log_{10}$cfu/ml for the wet season and 0$\log_{10}$cfu/ml. Acha samples had 2.39794 $\log_{10}$cfu/ml for the wet season and 1.56 $\log_{10}$cfu/ml for the dry season. The streams (Nkwo and Dorogo) complied with the SON and the WHO standards of 0 $\log_{10}$cfu/ml for drinking in the dry season only.

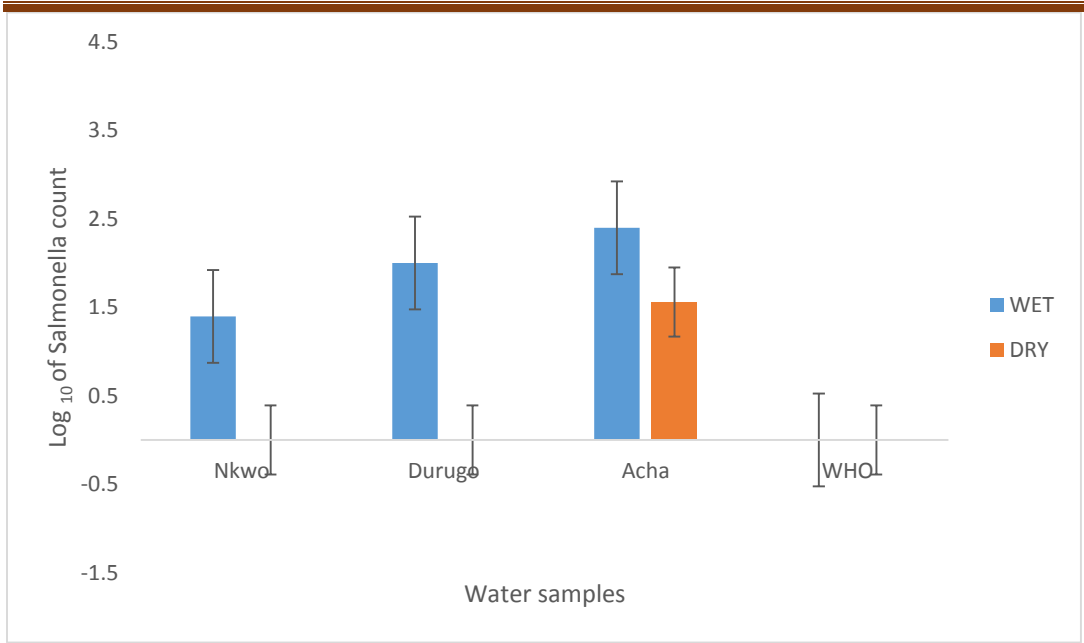

Figure 5.0: The Mean value for *Salmonellae* Counts of the Surface Waters in Nsu for the Wet Season and Dry Season.

***Shigella* counts**

Figure 6.0 illustrates the results for the seasonal variations in the *Shigella* counts of the three streams in Nsu community. The two streams (Nkwo and Dorogo) samples had 0log$_{10}$cfu/ml all the seasons. Acha recorded 1.477121 log$_{10}$cfu/ml during the wet season and 1 log$_{10}$cfu/ml in the dry season.

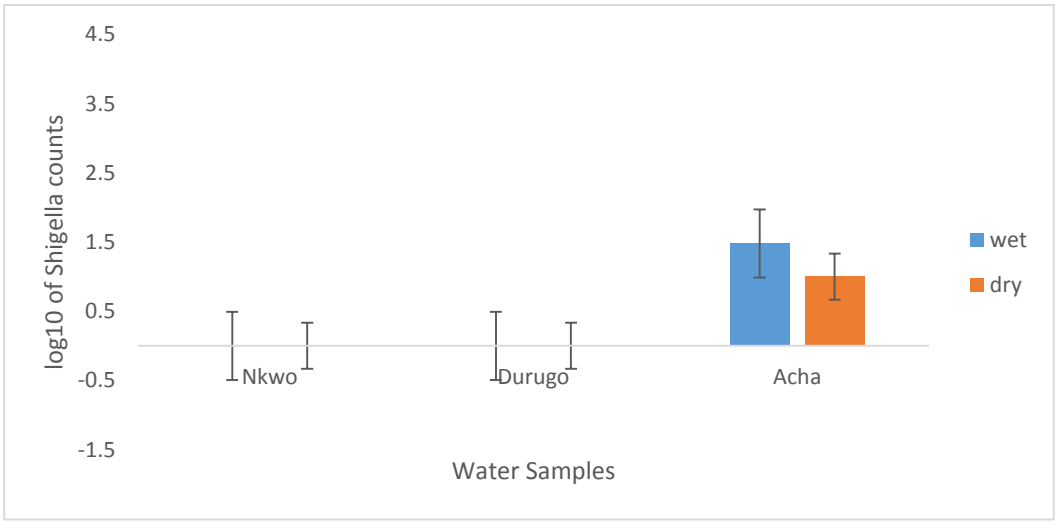

Figure 6.0: The Mean value for *Shigella* Counts of the Surface Waters in Nsu for both Wet Season and Dry Season

***Vibrio* counts**

Figure 7.0 illustrates the results for the seasonal variations in the *Vibrio* counts of the three streams in Nsu community. The two streams (Nkwo and Dorogo) samples had 0 log_{10}cfu/ml all the seasons. Acha samples recorded 1.47712 log_{10}cfu/ml during the wet season and 1 log_{10}cfu/ml in the dry season.

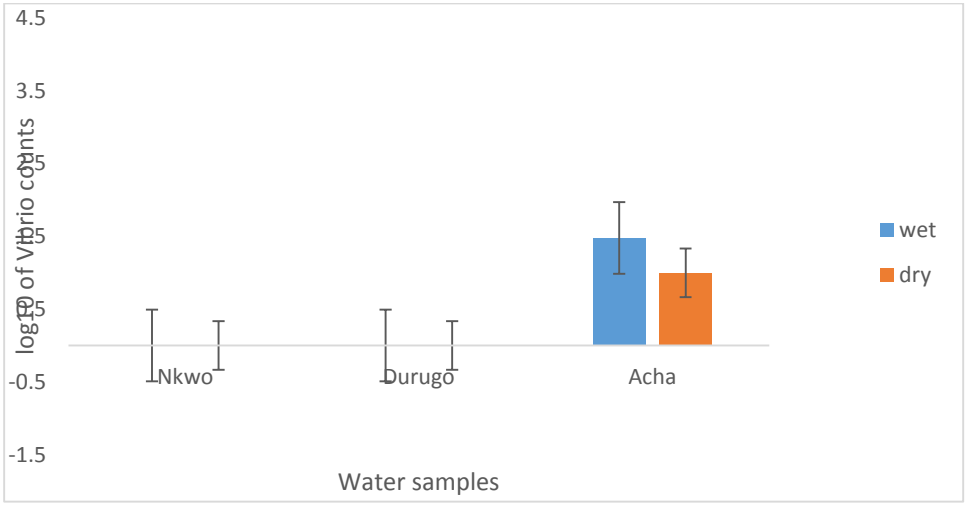

Figure 7.0: The Mean value (Log_{10}) for*Vibrio* Counts of the Surface Waters in Nsu for the Wet season and Dry season

Bacteria that were unable to use CO_2 as their sole source of carbon are called heterotrophic bacteria. Heterotrophic bacteria compete with their host for the reduced forms of carbon. The total heterotrophic bacterial counts for the three streams (Nkwo, Dorogo and Acha) water samples in the dry season are: for Nkwo water samples 3.249198 log_{10}cfu/ml, for Dorogo water samples 3.30103 log_{10}cfu/ml, for Acha recreational stream water samples 3.645913 log_{10}cfu/ml. This work is in agreement with Anazo and Ibe [10] who got a much higher THBC 5.1139log_{10}cfu/ml from a recreational water sources in the same geographical location and the same season. The results for the total heterotrophic bacterial counts for the dry season are: for Nkwo and Dorogo drinking water samples 3.070038 log_{10}cfu/ml and 3.164353 log_{10}cfu/ml respectively. Acha recreational water samples had 3.406540 log_{10}cfu/ml. These results are in agreement with WSAA [11] who carried out prospective studies on different water sources and saw changes in the results of their total heterotrophic bacterial count, total coliforms and faecal coliform counts as a result of seasonal changes. The total heterotrophic bacterial counts for the

drinking water sources and recreational water sources are higher than the SON and the WHO [12] permissible limits of 2 $\log_{10}$cfu/ml. The increase in the THB counts of the three streams can be attributed to pollution due to flood, runoff and waterlog which increases progressively with the volume of rainfall. The decrease in the THB counts of the three streams during the dry season can be attributed to lack of rainfall during these months which reduced the level of organic loads and controlled leaches and eutrophication. These findings agree with similar study by other workers who reported that the source of heterotrophic bacteria are human and animal wastes, run off, pastures, natural soil or plant bacteria, sewage and other unsanitary practises [13, 14].

The smallest most probable number (MPN) values for the total coliforms in the drinking water samples are: for Nkwo is 19.75MPN/100ml and for Dorogo is 21.75MPN/100ml, while that of the recreational water sample is 33.5MPN/100ml. These values are far above the SON and WHO limits for water. These results are also a serious indication that the water samples were faecal contaminated [15].This result is in total agreement again with the finding by Nwachukwu and Ume [16] who studied different water sources and found that 100% of the water sample from the stream all recorded above 10 MPN/100 ml.

The presence of *E. coli*, *Klebsilla*, *Enterobacter*, *Salmonell*a, *Shigella* and *Vibrio* species and other pathogenic bacteria not only make the water unsuitable for man but also pose serious health concerns [17]. Similar studies reported the presence of these organisms in both drinking and recreational sources [18, 19] and attributed it to indiscriminate disposal of organic wastes and contamination of these water sources by faeces through seepages and flood during the wet season. The bacterial microflora isolated from the water sources in Nsu community revealed predominance of the Gram negative bacteria up to 80% over the Gram positives 20%. This agrees with findings of Ibiene *et al* [20] who recorded 80% to 20% Gram negative to Gram positive dominance respectively from drinking water sources in Opuruaja community in Delta State, Nigeria. The dominance of the Gram negative bacteria could be linked to the ability of the Gram negative bacteria to go into the state of viable but not culturable VBNC in the case of pristine conditions in these waters.

Some bacteria like Salmonella sp., *Shigella*s p., *Vibrio* sp., *E. coli*, *Citrobacter* sp, *Klebsiella* sp., *Bacillus* sp., and *Enterobacter* sp., were all isolated from the water samples. The isolation of these organisms from these streams is a serious indication of potential diseases and epidemics.

This is because most of these bacteria are pathogenic, while the remaining ones are regarded as opportunistic pathogen that causes their infections in immune-compromised individuals [21].

REFERENCES

1. Douglas, S. I. & Isor, F. N. (2015). Bacteriological Investigation of Pond Water Quality from Ogoniland, Nigeria. *Journal of Environmental Science, Toxicology and Food Technology* (IOSR-JESTFT), 9 (2), 36-41
2. Figueras, M.J., Robertson, W. E. & Borrego, J.J. (2000). Sanitary inspection and microbiological water quality. *A Practical Guide to the Design and implementation of Assessments and Monitoring Programmes.* 113-167.
3. Edema, M.O., Omemu, A.M. & Fapetu, O.M. (2001). Microbiology and physiochemical Analysis of different sources of drinking water in Abeokuta Nigeria. *Nigerian Journal of Microbiology*, 15, 57-61.
4. WHO (1986).*Guidelines for Safe Recreational Water Environments*. Vol 2: *Swimming Pools and Similar Environments. 2004:* World Health Organization, Geneva.
5. Wolman, A. (1973). APHA in its first century. *Am J. Public Health*. 63:319-321.
6. Department of Geography and Environmental Management Studies, University of Port Harcourt, Nigeria
7. APHA, (2005). *Standard Methods for the Examination of Water and Waste Water*. 20th edition. American Public Health Association, Washington, DC, USA.
8. Cheesbrough, M. (2004). District Laboratory Practice in Tropical Countries, Part 1, LP ed. Cambridge University Press, London. Current Drinking Water Standards. USEPA, Washington DC.
9. Buchanan, L.E. & Gibbons, M.E. (1980). Bergey is manual of determinative bacteriology, Baltimore, the William and Willing Sco.
10. Anazo, I.J. &d Ibe, S.N. (2004). Sanitary Quality of Ulasi River, Okija, Anambra State. *African Journal of Applied Zoology and Envrionmental Biology*. 7: 52-55
11. WSAA (2003). Catchments for Recreational water: *Conducting and Assessing Sanitary Inspections* Occasional paper NO.8Water Services Association of Austrailia.
12. World Health Organisation (2008). *Guidelines for Drinking- Water Quality*. 3rdedition . World Health Organisation; Geneva, Switzerland.

13. Ibe, S.N. and Okplenye J.I. (2005). Bacteriological analysis of borehole water in Uli, Nigeria. *African Journal of Applied Zoology and Environmental Biology.* 7,116-119.
14. Kiman-murage, E.W. & Ngindu, A.M. (2007). Quality of water the slum dwellers use: the case of a Kenyan slum. *Journal of Urban Health*, 220-222.
15. Ajayi, A.O. & Akonai, K.A. (2005). Distribution pattern of enteric organisms in the lagos lagoon. *African Journal Biomedical Resources*, 8 (3), 163-168.
16. Nwachukwu, E. & Ume, C. A. (2013). Bacteriological and Physicochemical Qualities of Drinking Water Sources in the Local Areas of Eastern Nigeria . *Journal of Environmental Science and Water Resources.* 2(9), 336-341.
17. WHO (2010). Guidelines for safe recreational water Environment volume 1 *Coastal and Fresh Waters*, World Health Organization, Geneva.
18. Okonko, I.O., Ogunjobi, A.A. Adejoye, O.D., Ogunnisi, T.A. & Olasogba, M.C. (2008). Comparative Studies and Microbial Assessment of Different water Samples used for processing frozen sea foods in Ijora-olpa, Lagos State, Nigeria. *African Journal of Biotechnology*, 7 (16), 2902-2907.
19. Adejuwon, J.O. & Adelakun, M.A.(2012). Physiochemical and bacteriological analysis of surface waters in Ewekoro Local Government of Ogun State, Nigeria: Case study of lala, yobo and Aoddo Rivers. *International Journal for water Resources and Environmental Engineering*. 4 (3), 66-73.
20. Ibiene, A.A., Agbeyi, E.V. & Okonko, I.O. (2012). Bacteriological assessment of Drinking water sources in Opuraja Community of Delta State Nigeria. *Nature and Science.*10 (1)
21. Prescott, L.M., Harley, J.P. & Klein, D.A. (2005). *Microbiology,* 6 ed. McGraw Hill Co, New York, USA.